Differential Gene Expression of Matrix Metalloproteinases (MMPs), MMP-inhibitors (TIMPs and RECK), and MMP-activator (EMMPRIN/ CD147) During Osteogenic Differentiation from Human Dental Pulp Stem Cells (DPSCs)

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ABSTRACT

Constant remodeling of extracellular matrix (ECM) is a hallmark during physiological conditions, such as stem cell differentiation, embryogenesis and tissue repair. Matrix metalloproteinases (MMP) play a key role in these processes. MMPs, MMP-activator (EMMPRIN/CD147) and MMP-inhibitors (TIMPs and RECK) are responsible for bone matrix remodeling and, probably, determinate the level of its turnover. Mesenchymal Stem Cells derived from dental pulp are multipotent and have the capacity to differentiate into several mesenchymal tissues, such as bone, fat and cartilage, under inductive conditions in vitro. However, it is unknow In this study, we evaluated differential gene expression of MMPs (25 members), TIMPs (4 members), RECK, and EMMPRIN/CD147 of dental pulp stem cells (DPSCs) exposed to osteogenic induction. DPSCs isolated from extracted human third molars (collagenase/dispase digestion at 37°C) were grown in α-MEM medium + 10% FBS and differentiation induction in presence of osteogenic medium (10 mM β-glycerophosphate, 1 µM dexamethasone and 50 µg/ml ascorbate) for 35 days. We measured bone formation markers (osteocalcin, alkaline phosphatase and mineral nodules) using western blot, colorimetric assay and Alizarin Red S dye, respectively, and gene expression by qRT-PCR. After osteogenic differentiation, bone formation markers, matrix mineralization and differential gene expression were observed. This is the first evidence that MMPs, TIMPs, RECK, and EMMPRIN/CD147 are differentially expressed in osteoblast differentiation from DPSCs in vitro.
Keywords: Dental pulp stem cells, MMP, TIMP, RECK, EMMPRIN/CD147, and Osteoblast Differentiation. Financial Support: FAPESP

MATERIALS AND METHODS

- Cell Culture: Normal human impacted third molar was collected from adult (32-years old) at the Dental Clinic of University Hospital at University of São Paulo under approved guidelines set by the National Institutes of Health Office of Human Subjects Research. The pulp tissue was gently separated from the crown and root and then digested in a solution of 3 mg/ml collagenase type I (Gibco) and 4 mg/ml dispase (Gibco) for 1 h at 37°C. Single-cell suspensions were obtained by passing the cells through a 70-µm strainer. Cells were cultured in α-MEM + 10% SFB + 100 µM L-ascorbic acid 2-phosphate (clonogenic medium), and then incubated at 37°C in 5% CO2 (Gronthos et al 2000).
-Differentiation Medium: osteogenic (α-MEM + 10% SFB + 1 µM dexamethasone + 10 mM β-glicerofhosfate + 50 µg/mL L-ascorbic acid 2-phosphate), adipogenic (α-MEM + 10% SFB + 100 nM dexamethasone + 5 µg/mL insulin + 200 µM indometacin + 100 µg/mL IBMX) and chondrogenic (DMEM:F12 + 500 ng/mL insulin + 1 µM dexamethasone + 50 µM L-ascorbic acid 2-phosphate+ 10 ng/mL TGF-β1 + 1mM sodium piruvate) Verification of differentiation was assessed by specific dyes: alizarin red (osteogenic differentiation), oil red (adipogenic differentiation) and toluidin blue (chondrogenic differentiation).
- Flow Cytometry (FACS): Cells were divided into FACS tubes at 1 × 10^6 cells/tube and stained with FITC, APC or PE- conjugated anti-CD14, -CD45, -CD105, -CD73, -CD90, - CD31, –CD29, -CD34, -STRO-1, -IgG1. After incubating 60 min at ambient temperature in the dark, cells were washed and supernatant was removed and cells were fixed with 1% formaldehyde (60 min). All data was acquired using a FACSAria II (BD) and analyzed using DIVA (BD) and Flowjo software (Treestar, Inc.).
- qRT-PCR: Total RNA was used as template for cDNA synthesis in a RT-PCR reaction and qRT-PCR reaction was performed by SYBR® Green Dye I (Applied Biosystems - 40 cycles at 60°C). Relative quantification was performed by Pfaffil method (2001). B-actin was used as housekeeping gene and undifferetiated cell as reference sample. GeNorm algorithm (Vandesompele et al., 2002) was used to determine the normalization factor.

RESULTS

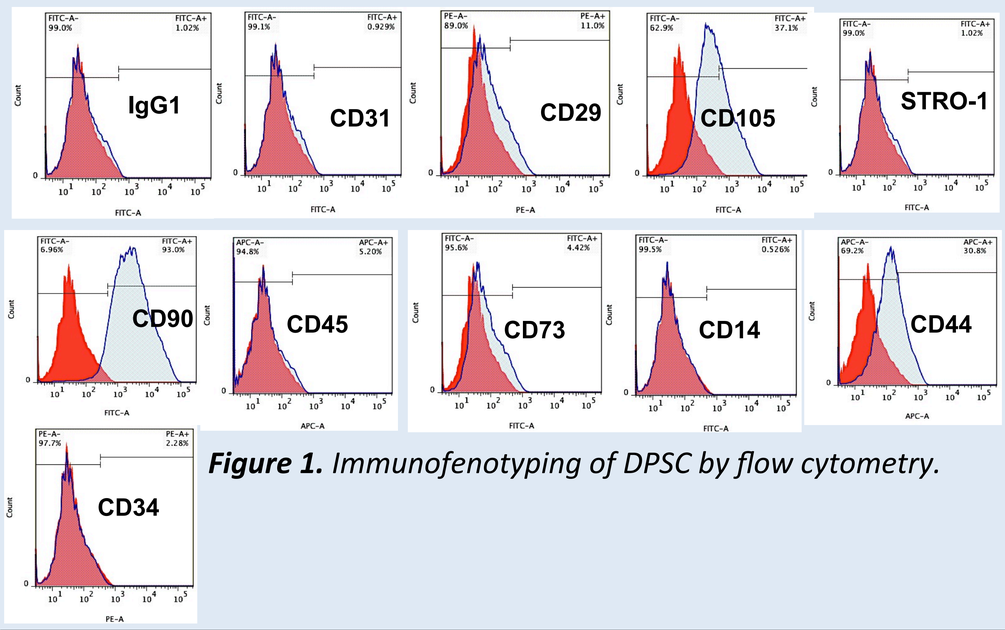


Figure 1. *Immunofenotyping of DPSC by flow cytometry.*

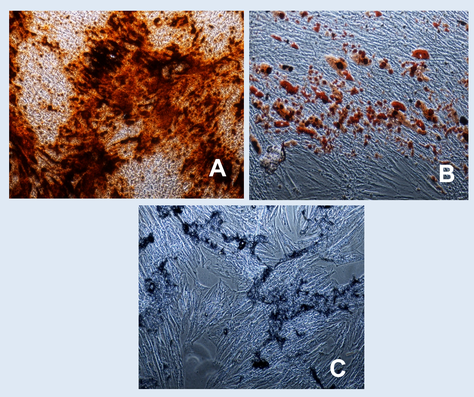


Figure 2. *Confirmation of multilenage differentiation from DPSC. (A) Alizarin red, (B) Oil red, (C) Toluidin blue.*

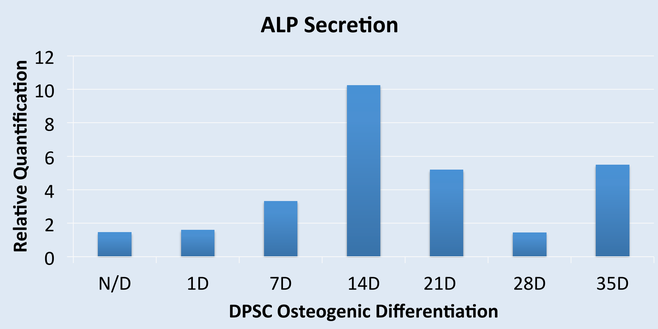


Figure 3. *Quantification of alkaline phosphatase secretion throughout osteogenic differentiation from DPSC*

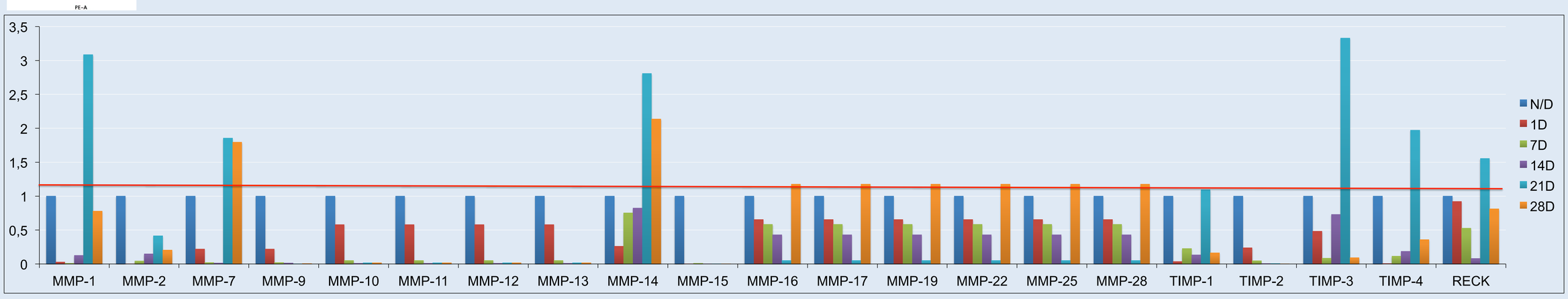
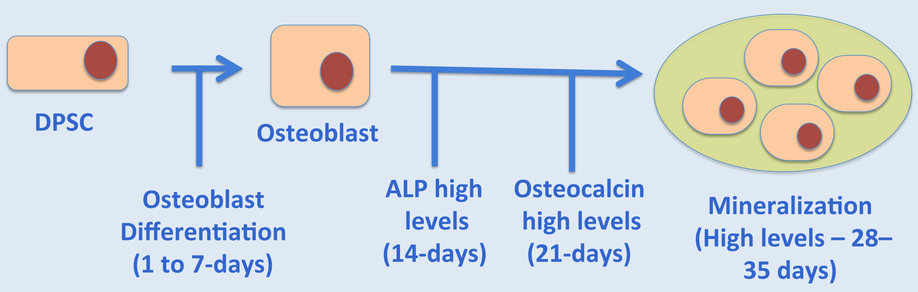


Figure 4. *Gene expression of MMPs, TIMPs, RECK and EMMPRIN after osteogenic differentiation induction from Dental Pulp Stem Cells evaluated by relative real-time PCR analysis (qRT-PCR). Results are relative to the normalized expression of housekeeping gene (B-actin). N/D (undifferentiated cell): reference sample. Down-regulated genes: EMMPRIN, MMP-2, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13, MMP-15, TIMP-2; Up-regulated genes: MMP-1 (21D), MMP-7 (21D and 28D), MMP-14 (21D and 28D), MMP-16 (28D), MMP-17 (28D), MMP-19 (28D), MMP-22 (28D), MMP-28 (28D), TIMP-1 (21D), TIMP-3 (21D), TIMP-4 (21D) and RECK (21D).*

DISCUSSION AND CONCLUSION



Taken togheter, MMP-1, MMP-14, MMP-7, TIMP-3, TIMP-4 and RECK may be important to mineralization because they were up-regulated at 21-days after osteogenic differentition induction. On the other hand, other genes were repressed in this process, such as EMMPRIN, MMP-2, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13, MMP-15 and TIMP-2. MMP-15, MMP-16, MMP-17, MMP-19, MMP-22, MMP-25, MMP-28 have the same expression profile, being down-regulated from 1D to 21-days andm apparently, starting of up-regulation at 28-days. The down-regulated genes may be important to stemness properties in DPSC.